Proc. World Maricul. Soc. 10:435-444 (1979)

MATURATION OF WHITE SHRIMP (*Penaeus setiferus*) IN CAPTIVITY[1]

A. Brown, Jr.[2], J. McVey[2], B. S. Middleditch[3], and A. L. Lawrence[4]

ABSTRACT

Penaeus setiferus was matured and spawned in captivity by using ambient temperature and controlling photoperiod and diet. The experiments were performed at the lagoon laboratory and the seawater laboratory of the National Marine Fisheries Service, Galveston, Texas. One-half of the animals at each site were unilaterally ablated. The photoperiod was initially set at 15 hours light and increased to 16 hours with the onset of spawning; the temperature was 22-29°C, salinity 22-30 ppt and pH 7.5-8.0. The diet consisted of worms, squid, oysters, and mussels. Egg production varied between the two sites, with 534,000 eggs produced at the lagoon facility and 3,800,000 eggs at the seawater laboratory. Spermatophore transfer did not take place, perhaps because of a bacterial infection (*Vibrio* sp.) of the terminal ampoules and the compound spermatophore.

INTRODUCTION

If marine shrimp farming is to become a viable industry the sexual maturation of shrimp in captivity must be achieved. Johnson and Fielding (1956) reported the first successful maturation and spawning (with fertilized eggs) of the white shrimp (*Penaeus setiferus*) in captivity. Recently Conte et al. (1977) matured *P. setiferus* in hypersaline ponds, but spawning did not occur. Other species of shrimp that have been matured in the laboratory include *P. duorarum* (Idyll, 1971; Caillouet, 1973), and *P. aztecus* (Duronslet et al., 1975), all using the technique of ablation to induce ovarian development. The SEAFDEC (Southeast Asian

---

[1]Contribution No. 79-26G.

[2]Galveston Laboratory, Southeast Fisheries Center, National Marine Fisheries Service, Galveston, TX 77550.

[3]Department of Biophysical Sciences, University of Houston, Houston TX 77004.

[4]Department of Biology, Program in Physiology and Marine Science, University of Houston, Houston, TX 77004. Present address: Texas Agricultural Experiment Station and Department of Wildlife and Fisheries Sciences, Texas A&M University, P.O. Box 1067, Corpus Christi, TX 78410.

Fisheries Development Center) maturation team (1976) has also successfully used ablation to induce ovarian development in *P. monodon*, producing viable eggs.

P. californiensis was matured and spawned in continuous flow systems by Moore et al. (1974), but fewer eggs were produced by captive *P. californiensis* than by wild stock. Aquacop (1975) matured and spawned *P. merguiensis* (two generations), *P. japonicus*, *P. aztecus* (unilateral ablation only), and *Metapenaeus ensis* in a continuously flowing system in a tropical environment. Laubier-Bonichon and Laubier (1976) and Laubier-Bonichon (1978) have achieved reproducible maturation of *P. japonicus* by strict control of environmental parameters, e.g., temperature, photoperiod and diet. In both *P. japonicus* and *P. merguiensis*, spawnings of the second generation have been achieved.

The ability to mature and spawn shrimp in captivity would enhance the industrial development of shrimp aquaculture by circumventing expensive trips offshore to collect mature shrimp, providing a reliable source of seed stock on a year-round basis and providing the opportunity to conduct genetic studies and selective breeding for improvement of the commercial qualities of shrimp.

The objective of the cooperative experiments conducted by the University of Houston, Texas A&M University, and the National Marine Fisheries Service* was to mature *P. setiferus* in captivity. *P. setiferus* was selected because it is indigenous to the Gulf of Mexico and has been reported to grow more rapidly than *P. aztecus* in pond culture (Parker and Holcomb, 1973). The photoperiod, temperature and diet regimes used in this study are reported here. Our objectives also included biochemical analysis of the food contained in the diet and *P. setiferus* as it approached maturation and spawning; these data are reported by Lawrence et al. (1979) and Middleditch et al. (1979).

MATERIALS AND METHODS

Adult *P. setiferus* were obtained by trawling near Freeport, Texas, June 12, 1978. The majority of the females did not show any signs of ovarian development.

Animals were stocked at a density of 6.5/m^2 into four raceways (8.5 x 1.2 m) at the NMFS lagoon laboratory (1:1 ratio male to female) and two 3.0 m dia tanks at the NMFS seawater laboratory. Both the raceways and circular tanks had continuous water flow with a turnover rate of 3 to 4 times a day. The initial photoperiod of 15 hours light was increased to 16 hours when the animals began to spawn; the temperature varied from 22-29°C and the initial salinity of 22 ppt rose to a high of 30 ppt (Fig. 1 and 2).

The diet consisted of marine worms (as suggested by Dr. Laubier-Bonichon, personal communication), *Glycera dibranchiata* (blood worms) and *Nereis virídens* (sand worms), squid (*Loligo* sp.), oysters (*Crassostrea* sp.) and mussels (*Mytilus edulis*). Mussels were later discontinued

---

*Maturation, NMFS: Dr. J. McVey, Division Chief; Aquaculture, Ausbon Brown, Jr., Project Leader, Bryce Scott, Theodore Williams, Marcel Duronslet, Rick Newitt, Pat Hammack, Norman Sears.

because of supply problems. The animals were fed four times daily at 3% body weight per day. Four feeding periods were established: 0800, worms; 1200 noon, squid; 1600, worms; 2000, oysters and mussels or mussels. These periods were established so that the quantity of food would not be limiting and to ensure that a variety of nutrients was supplied during different times of the day.

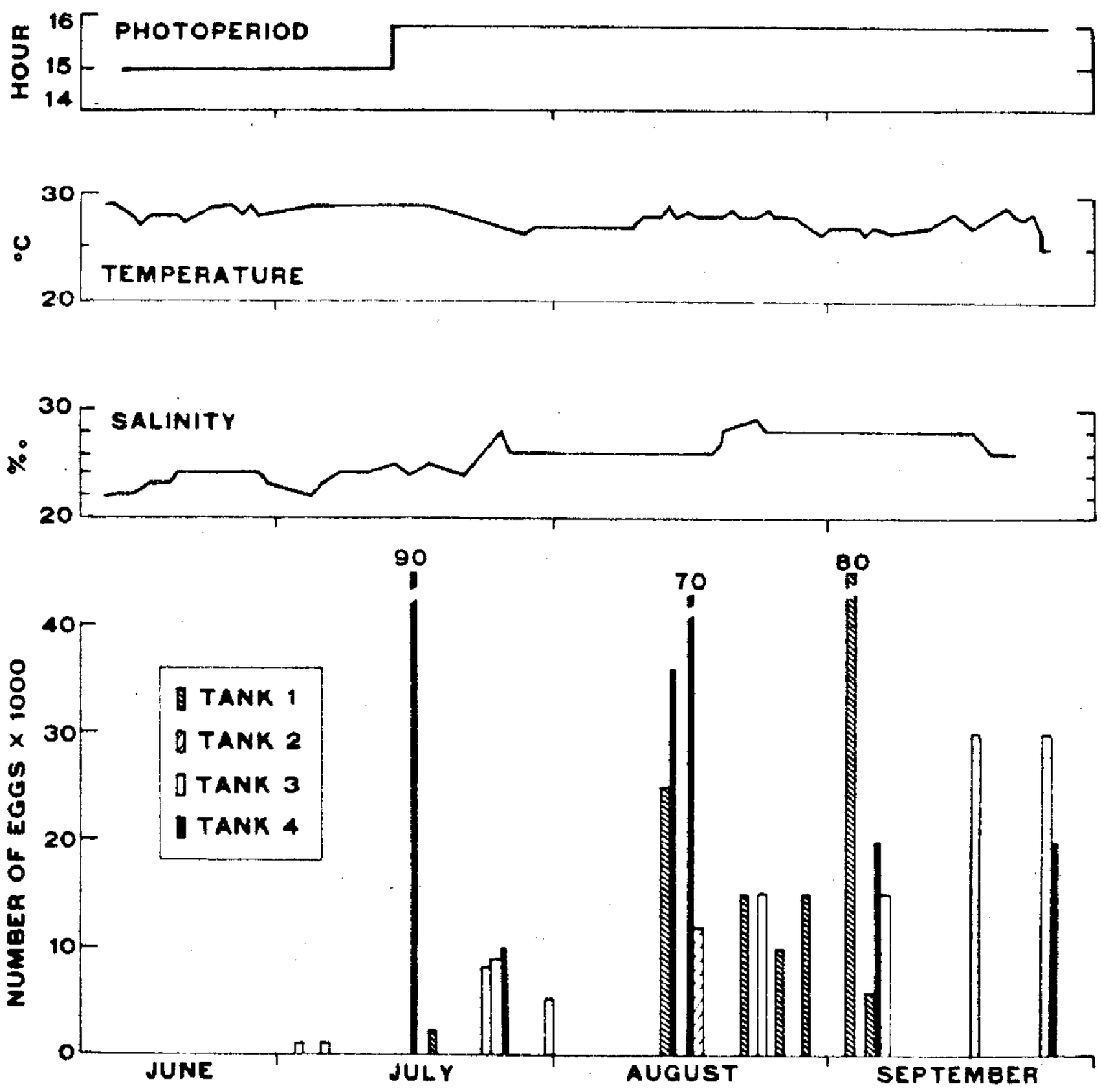

Figure 1. Graph showing the interaction of temperature, salinity, photoperiod and spawning activity at NMFS lagoon facility.

The worms were fed twice daily because of their ready acceptability by the shrimp and the presence of long-chain fatty acids thought to promote ovarian development (Middleditch et al., 1979). Squid was fed at midday because it was consumed more slowly and any excess could be removed at the next feeding (1600 hours). The mussels on the half-shell were better for the final feeding because they did not foul the tank. Mussels thrown into the tank invariably landed on the bottom face up. The mussel flesh never touched the bottom of the tank, and the shrimp cleared the shells of all flesh, leaving only the shells to be retrieved. Mussels were much more acceptable as food than oysters; however, oysters were added to the diet when supply problems developed with mussels.

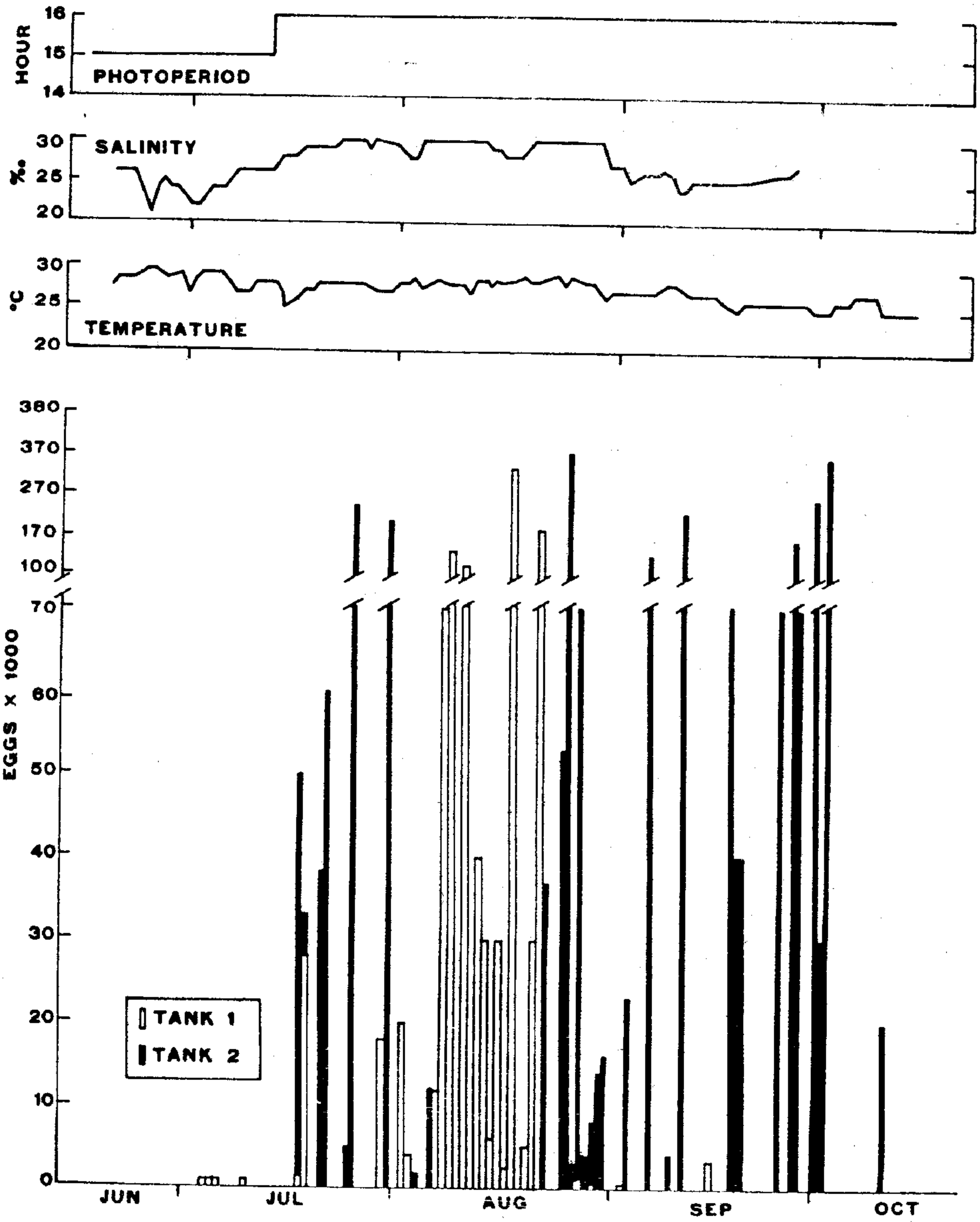

Figure 2. Graph showing the interaction of temperature, salinity, photoperiod and spawning activity at NMFS seawater laboratory.

Both the raceways and the circular tanks were equipped with egg collectors, similar to those of Laubier-Bonichon (1978). These collectors minimized disturbance of the animals and were checked daily for eggs as an indication of spawning. Eggs were collected between 0800 and 1000 hours and during the same period the tanks were cleaned of waste material and excess food. No assessment could be made as to the number of times individual females spawned.

One-half of the animals at each site were unilaterally ablated by the method of Primavera and Borlongan (1977). A small incision was made in the eyeball and the material of both eye and eyestalk expressed. The temperature was reduced to approximately 29°C before ablation to minimize activity and reduce possible loss of hemolymph.

A second experiment was started on November 29, 1978, to determine if ovarian maturation could be induced using these methods when *P. setiferus* was not normally maturing and spawning offshore.

RESULTS

P. setiferus adapted well to both raceways and circular tanks without mass mortality. However, the shrimp reacted to the white color of the raceways and the circular tanks by swimming very closely to the walls and rubbing their eyes against the sides of the tank. Shrimp in the blue raceways exhibited the same behavior to a lesser extent.

Ovarian development was observed approximately 3 to 4 weeks after the animals were introduced into experimental tanks (see Table 1 for dates of initial collections). There were 63 egg collections resulting in 4,335,000 eggs. A total of 534,000 eggs were produced by animals held at the lagoon laboratory (raceways) and 3,800,000 eggs were produced by animals in the seawater laboratory (circular tanks). The spawning activity and number of eggs collected per day were greater in the seawater laboratory than in the lagoon laboratory. The salinity was initially lower and in general more variable for the lagoon as opposed to the seawater laboratory (Fig. 1 and 2).

TABLE 1. Initial Spawnings: *Penaeus setiferus*

	Initial collection date	No. of eggs
Lagoon laboratory		
Raceway #1	7/19/78	1,000
Raceway #2	8/16/78	12,000
Raceway #3	7/04/78	1,000
Raceway #4	7/17/78	104,000
Seawater laboratory		
Circular tank #1	7/04/78	1,000
Circular tank #2	7/05/78	1,000

Unablated shrimp spawned as many eggs as the ablated animals at the lagoon laboratory: 274,000 and 262,000 eggs, respectively (Table 2). However, in the seawater laboratory the unablated animald did not produce as many eggs as the ablated animals (1,095,000 and 2,706,000 eggs, respectively). Further, the 50 females in the circular tanks at the seawater laboratory produced at least seven times the number of eggs produced by 132 animals at the lagoon laboratory.

TABLE 2. Production of Eggs by Ablated and Unablated Female *Penaeus setiferus* Maintained in Laboratory Tanks

	No. of eggs	Ablated
Lagoon laboratory		
Raceway #1 and #3	262,000	Yes
Raceway #2 and #4	274,000	No
Seawater laboratory		
Circular tank #1	1,095,000	No
Circular tank #2	2,706,000	Yes

None of the eggs produced during these experiments was fertilized. Some of the females were sacrificed to determine if the eggs were capable of undergoing the cortical reaction and hatching membrane formation described by Clark et al. (1975). The ovaries were dissected and placed in seawater and viewed with the microscope. The majority of the eggs were observed to undergo the cortical reaction and formation of the hatching membrane.

A second experiment using the method described above began November 28, 1978. This is significant because it represents induction of ovarian maturation at a time when the natural populations are not maturing. This indicated that a reproducible method for maturing *P. setiferus* in captivity will be achieved once the problem of spermatophore transfer has been solved.

DISCUSSION

It is apparent that the experimental conditions and diet reported in this study were sufficient to induce ovarian development in *P. setiferus*, but it is unclear why spermatophore transfer and fertilized eggs were not obtained. The majority of the eggs were capable of underoing the cortical reaction and hatching membrane formation and thus probably capable of being fertilized. In comparison to other male penaeid shrimp used in similar studies (Aquacop, 1975), the male *P. setiferus* were morphologically capable of spermatophore transfer, and mature sperm were contained in their compound spermatophores dissected from male *P. setiferus* used in this study.

There were a number of possible reasons for failure of spermatophore transfer in *P. setiferus* during our experiments. Among these were the general health of the shrimp, the behavior of the shrimp toward the white and blue colors of the raceways and circular tanks, and the environmental conditions.

Penaeus setiferus reacted to the white of the raceways and circular tanks by swimming into the walls of the tanks severely damaging the eyes, antennular flagella, antennules, antennae and antennal scales. The damage to animals held in the blue raceways was less severe in comparison to those in white tanks. It could not be determined whether the behavior of the animals was altered sufficiently to prevent spermatophore transfer. At the conclusion of these experiments, all tanks were painted black. The animals in the second experiment now swim close to

the walls of the tank without injury.

The environmental variability during these experiments may have affected egg production and spermatophore transfer at the lagoon and seawater laboratories. The seawater laboratory afforded better control of environmental parameters than the lagoon laboratory (Fig. 1 and 2). This may account for the increased production of eggs at the seawater laboratory (almost 7 to 1) as compared to the lagoon laboratory. In addition, ablated animals in the seawater laboratory yielded almost 10 times more eggs than the ablated animals held at the lagoon laboratory, suggesting that environmental conditions were better at the seawater laboratory than at the lagoon laboratory. This effect of environmental factors observed with egg production may have also contributed to failure of spermatophore transfer. These conditions may not have been optimum even though maturation and spawning occurred with females.

Perhaps the most likely cause of failure of male *P. setiferus* to transfer the spermatophore was a bacterial infection (*Vibrio* sp.) of the terminal ampoules and the enclosed spermatophores. The infection appeared at the midpoint of the experiment. The damage to the compound spermatophore was extensive, with complete destruction of the wings, geminate body and flanges, making it impossible for the spermatophore to become attached during mating. This condition was observed in all males at the conclusion of the experiment.

It is well known that it is better to maintain the relative constancy of environmental parameters when maturing shrimp in captivity (Laubier-Bonichon and Laubier, 1976; Moore et al., 1974; Aquacop, 1975). However, the literature is very limited regarding these factors in *P. setiferus* maturation and spawning and any effects that these parameters may have on the maturation process and spermatophore transfer. The same is true for *P. schmitti*, *P. occidentalis*, *P. vannaemi* and *P. stylirostris*. These shrimp possess an open thelycum, lack a seminal receptacle and are non-grooved and non-burrowing (Farfante, 1975). The males of this group transfer the spermatophore after ovarian development and just before spawning. The spermatophore does not remain attached and it is easily dislodged (Hanson and Goodwin, 1977). Biologists collecting mature white shrimp for spawning have found that along with ovarian coloration (Brown and Patlan, 1974) some evidence of spermatophore transfer (e.g., sperm mass or spermatophore) must be found, otherwise unfertilized eggs were spawned. These characteristics of the white shrimp may account for the fact that the majority of efforts in shrimp maturation have been directed toward brown or grooved shrimp. The shrimp belonging to this group are characterized by their rostral grooves, burrowing habit, closed thelycum and a seminal receptacle. Spermatophore transfer in these animals takes place before ovarian development and sperm are carried until the animals molt or until the ovaries develop and spawning takes place (Aquacop, 1975; Laubier-Bonichon and Laubier, 1976; Laubier-Bonichon, 1978; Moore et al., 1974; Aquacop, 1976; SEAFDEC, 1976; Santiago, 1977).

Therefore, the majority of shrimp that have been matured and spawned in captivity have been those penaeid shrimp possessing the closed thelycum and morphological capability of storing sperm. The females of this group are fertilized before ovarian development (Aquacop, 1977) with ovarian maturation and spawning occurring later. Spermatophore transfer in these shrimp decreases the prospect of obtaining unfertilized eggs and also the effects of environmental parameters on spermatophore transfer because the ovaries develop with sperm already present.

The only non-grooved shrimp possessing an open thelycum that has been matured and spawned successfully producing viable eggs is *P. stylirostris* (Aquacop, 1977). If we contrast the environmental requirements and spermatophore morphology of *P. stylirostris* (tropical species) with *P. setiferus* (temperate species), it may aid in understanding why maturation and spawning experiments have been successful with *P. stylirostris* but not *P. setiferus*.

P. stylirostris is found in the tropics and is subject to very little environmental fluctuation so that a strict temperature and photoperiod sequence may not be necessary for successful maturation. Therefore, diet may be one of the few limiting factors. This is not true with *P. setiferus* because in its temperate location there is greater difference in the seasons, which may provide conditioning for the maturation process. To reproduce these environmental changes requires greater control in the experimental process.

Spermatophore transfer probably takes place after ovarian development in *P. stylirostris* as in *P. setiferus*; however, spermatophore attachment is more secure in the former due to the elaborate development of the coxal plates of the fourth and fifth peroipods, which are involved in attachment. Furthermore, part of the ventral and lateral walls along with the wings of the spermatophore provide protection for the sperm mass once spermatophore transfer has taken place (Farfante, 1975). It appears then that *P. stylirostris* is very similar to shrimp possessing a closed thelycum, in terms of the firm attachment of the spermatophore and the shielding of the sperm from being dislodged, assuring that sperm will be present when spawning occurs.

In this experiment with *P. setiferus*, ovarian maturation and spawning were achieved, but environmental conditions, the physical characteristics of holding tanks, or a bacterial infection may have prevented spermatophore transfer and the attainment of fertilized eggs. In contrast, the animals possessing a closed thelycum have been matured and spawned on many occasions, resulting in fertilized eggs.

In conclusion, this paper represents the first report of successful maturation with spawning of a temperate, non-grooved and non-burrowing penaeid shrimp in tanks under laboratory conditions. Johnson and Fielding (1956) reported maturation of *P. setiferus*, but their work was in ponds. We feel that in attempting to mature shrimp in captivity that different procedures will have to be used depending upon whether the species has an open or closed thelycum, mating before or after ovarian maturation, a temperate or tropical natural environment and whether the shrimp are burrowing or non-burrowing.

ACKNOWLEDGMENTS

We thank all the members of the maturation team of the National Marine Fisheries Service for their support and thoughtful suggestions. Also, we thank Mr. Hoyt C. Holcomb and Mr. David Ramsey for their suggestions and for sourcing *Penaeus setiferus*. This work is a result of a research program sponsored in part by Texas A&M University Sea Grant College Program, supported by the National Oceanic and Atmospheric Administration, Office of Sea Grant, Department of Commerce, under Grant No. 4-7-158-44105.

LITERATURE CITED

Aquacop. 1975. Maturation and spawning in captivity of penaeid prawns *Penaeus merguiensis* de Man, *Penaeus japonicus* Bate, *Penaeus aztecus* Ives, *Metapenaeus ensis* de Hann, and *Penaeus semisulcatus* de Haan. Proceedings World Mariculture Society 6:123-132.

Aquacop. 1976. Reproduction in captivity and growth of *Penaeus monodon* Fabricius in Polynesia. Centre Oceanologique de Pacifique, CNEXO-COP, Taravao-Tahiti (French Polynesia). An Internal Report, pp. 1-20.

Aquacop. 1977. Observations on the maturation and reproduction of penaeid shrimp in captivity in a tropical medium. Pages 1-34 in Aquaculture Workshop ICES, May 10-13, Brest, France.

Brown, A., and D. Patlan. 1974. Color changes in the ovaries of penaeid shrimp as a determinant of their maturity. Marine Fisheries Review 36(7):23-26.

Caillouet, C. W., Jr. 1973. Ovarian maturation by eyestalk ablation in pink shrimp *Penaeus duorarum* Burkenroad. Proceedings World Mariculture Society 3:205-225.

Clark, W. H., A. Yudin, W. J. Lynn, and A. Persyn. 1975. The cortical reaction in the eggs of the penaeid shrimp. Presented at International Symposium of Physiology of Reproduction in the Invertebrates, Calicut University.

Conte, F. S., M. S. Duronslet, W. H. Clark, and J. C. Parker. 1977. Maturation of *Penaeus stylirostris* (Stimpson) and *P. setiferus* (Linn.) in hypersaline water near Corpus Christi, Texas. Proceedings World Mariculture Society 8:327-334.

Duronslet, M. J., A. I. Yudin, R. S. Wheeler, and W. H. Clark, Jr. 1975. Light and fine structural studies of natural and artificially induced egg growth of penaeid shrimp. Proceedings World Mariculture Society 6:105-122.

Farfante, I. P. 1975. Spermatophores and thelyca of the American white shrimps, genus *Penaeus*, subgenus *Lito penaeus*. Fishery Bulletin 73(3):463-486.

Hanson, J. A., and H. L. Goodwin. 1977. Shrimp and Prawn Farming in the Western Hemisphere: State of the Art Reviews and Status Assessment. Dowden, Hutchinson and Ross, Inc., Stroudsburg, Pa. 439 pp.

Idyll, C. P. 1971. Induced maturation of ovaries and ova in pink shrimp. Commercial Fisheries Review 33(4):20.

Johnson, M. C., and J. R. Fielding. 1956. Propagation of the white shrimp, *Penaeus setiferus* (Linn.), in captivity. Tulane Studies in Zoology 4(6):175-190.

Laubier-Bonichon, A., and L. Laubier. 1976. Controlled reproduction of *Penaeus japonicus* shrimp. Pages 1-6 in FAO Technical Conference on Aquaculture, Kyoto, Japan, 26 May-2 June.

Laubier-Bonichon, A. 1978. Ecophysiology of reproduction in the prawn *Penaeus japonicus* three years experiment in control condition. Oceanologica Acta 1:135-150.

Lawrence, A. L., D. Ward, S. Missler, A. Brown, J. McVey, and B. S. Middleditch. 1979. Organ indices and biochemical levels of ova from penaeid shrimp maintained in captivity versus those captured in the wild. Proceedings World Mariculture Society 10:this volume.

Middleditch, B. S., S. R. Missler, D. G. Ward, J. P. McVey, A. Brown, and A. L. Lawrence. 1979. Maturation of penaeid shrimp: dietary fatty acids. Proceedings World Mariculture Society 10: this volume.

Moore, D. W., C. W. Sherry, and F. Montanex. 1974. Maturation of *Penaeus californiensis* in captivity. Proceedings World Mariculture Society 5:445-450.

Parker, J. C., and H. W. Holcomb. 1973. Growth and production of brown and white shrimp (*Penaeus aztecus* and *Penaeus setiferus*) from experimental ponds in Brazoria and Orange counties, Texas. Proceedings World Mariculture Society 4:215-235.

Primavera, J. H., and E. Borlongan. 1977. Notes on induced maturation and spawning in four month old *Penaeus monodon* Fabricius by eyestalk ablation. Contribution No. 10 of the Aquaculture Department, Southeast Asian Fisheries Development Center, Iloilo, Philippines.

Santiago, A. C., Jr. 1977. Successful spawning of cultured *Penaeus monodon* after eyestalk ablation. Aquaculture 11(3):185-196.

SEAFDEC. 1976. Development of a brood stock of the tiger prawn *Penaeus monodon* Fabricius. Technical Report No. 1, SEAFDEC.